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Production of Flavor in Cured Meat by a Bacterium

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The bacteriology of meat curing brines has been studied chiefly from the standpoint of preservation of meat. Such investigations have established that concentrations of salt used are not bactericidal but inhibitory to most bacteria, and that certain osmophilic bacteria and yeasts tolerate or, in some cases, even require salt concentrations for maximal growth.

The relationship of brine microorganisms to flavor production in the curing process has been mentioned from time to time by workers dealing with the chemistry and bacteriology of curing brines. This work has been reviewed by Tanner (1944) and Jensen (1954). Some workers have obtained evidence that seemed to indicate that microorganisms in brines were a factor in flavor production and some have obtained results to the contrary. Gibbons (1939) stated, "Little information can be found on the role bacteria actually play in meat curing." Brooks *et al.* (1940) reported that bacterial activity in curing brines was not essential to the production of satisfactory flavor in bacon and ham. Deibel and Niven in 1957 reported experiments in which pure cultures of lactobacilli and also mixed flora inocula were added to commercial curing brines in an effort to enhance flavor production. Bacterial growth was inhibited in control brines by the use of antibiotics. Evidence for the production of an undesirable sour flavor in the inoculated hams was obtained.

Fermentation by bacteria and yeasts plays an important part in the preparation of wines, cheeses, butter, molasses, pickles, sauerkraut, olives, and fermented sausages. Numerous references to detailed studies for the selection and use of microorganisms for the production of flavor and aroma in the products mentioned are to be found.

This work was undertaken to investigate the question as to whether or not the growth of a bacterium in a meat curing brine would affect flavor of the meat. The effects on flavor of a single bacterial species acting upon a substrate, obtained in sterile condition without heat or chemical treatment of the actual meat used, have been recorded. In this respect, there are inherent advantages which, to our knowledge, have not been extensively explored in the field of food fermentation studies.

MATERIALS AND METHODS

Hogs supplying hams for all experiments were of approximately the same age and weight and from several inbred lines developed at Beltsville in which Danish Landrace was crossed with various breeds. They had been maintained on standard diets in a record of performance study. The hams which had been held at 38 F from 2 to 7 days post slaughter were washed in hot water and detergent, Sparkleen[1] (1 tablespoonful per gallon), surface disinfected with Zephiran[2] by mopping the entire surface twice at an interval of 10 min with a 1:1000 solution in 80 per cent ethyl alcohol. Chunks of meat weighing approximately 50 g each were aseptically removed from the biceps and placed in a pint fruit jar of brine. Sterile, glass beads were added to raise the level of brine over the meat. A single muscle, the biceps, was used in each of the three experiments as different muscles of the same hog are known to differ in chemical composition (Brady *et al.*, 1944) and different beef muscles from comparable animals have been shown to differ in aroma, flavor, juiciness, and tenderness (Jacobson and Fenton, 1956). Half of the chunks from each ham were used for controls and half for testing the effect of added inoculum.

Brine contained 6 per cent sodium chloride, 1.5 per cent sucrose, 0.09 per cent sodium nitrate, and 0.009 per cent sodium nitrite. Sixty ml of the brine were used to cure a 50-g meat sample. These proportions are approximately the same as used in commercial and farm curing procedures. It was necessary to add all the nitrogen in the form of nitrite in control samples which lacked the presence of nitrate reducing bacteria. This was done to have the meat in both cases a desirable red color at the end of the curing period.

Bacterium. The test organism used (laboratory designation, HB28) was a gram negative rod displaying monotrichate and amphitrichate polar flagellation. The bacterium is slowly proteolytic at 33 C. It is capable of growing on Bacto-fluid thioglycolate[3] medium as well

[1] Sparkleen contains sodium hexametaphosphate. Fisher Scientific Company, Pittsburgh, Pennsylvania. Mention of specific commercial materials and equipment throughout this paper does not constitute recommendation for their use above similar materials and equipment of equal value.

[2] Active ingredient: benzalkoniumchloride. Winthrop Laboratories, New York, New York.

[3] Difco Laboratories, Inc., Detroit, Michigan.

as aerobically. It grows over the range of 0 to 41 C, utilizes ammonium salts as a sole source of nitrogen, is catalase positive, and reduces nitrates to nitrites. It is indole and H_2S negative. Acid is produced from glucose, sucrose, glycerol, mannitol, and maltose. It is not capable of growth on mineral agar containing ethanol and calcium carbonate (Stanier, 1947) and does not produce detectable amounts of acetic acid when grown at 30 C in enriched media to which ethanol has been added. The organism grows well in media containing 10 per cent NaCl, but better in 5 per cent NaCl. It grows poorly in media containing 0.5 per cent NaCl and does not exhibit the metabolic activities here recorded. These were determined on media containing 5 per cent NaCl. The only exception to this statement is noted in the ability of the bacterium to produce acid from sucrose in medium containing 0.5 per cent NaCl. Rapid formation of a water soluble brown pigment served as a marker in experimental work. The authors consider the bacterium a species of *Pseudomonas*. It is, apparently, closely related to *Pseudomonas nigrifaciens* but differs from it in several characteristics, particularly with respect to its reaction on sugars. It was selected for use in these experiments in flavor production by screening 35 cultures isolated from ham curing brines. A number of discriminating panel members indicated that meat cured in the presence of this bacterium possessed desirable flavor in comparison to meat cured in sterile brine or in brines plus other test organisms. Two human volunteers repeatedly drank without ill effects 10-ml quantities of brine from experimental cures in which the organism had grown for 72 hr. Inoculum for addition to experimental cure jars was prepared by plating an actively growing culture (three daily transfers immediately before use to seed Kolle flasks) 18 to 20 hr prior to use on Bacto veal infusion agar containing 6 per cent sodium chloride (the concentration of experimental brine). Growth was washed off in 6 per cent sodium chloride solution and diluted to Klett scale reading of 900, and 1 ml of this was added to each curing jar in the treated series.

TABLE 1

Results of triangular taste tests showing influence of Pseudomonas sp. strain HB28 in curing brine

Expt No.	No. of Tasters*		
	Total	Correct	Needed for 0.001 level of significance†
	Brine—Inoculated vs Control‡		
1	29	19	19
2	29	20	19
3	25	16	17
	Meat Extract—Inoculated vs Control		
1	29	23	19
2	27	18	18
3	26	18	17
	Meat—Inoculated vs Control		
1	26	19	17
2	27	20	18
3	27	19	18

* Total number of tasters for different materials in a given experiment varied as different materials were tasted on three successive days. It was not always possible to locate all judges.

† Roessler *et al.*, 1948.

‡ Taster given two inoculated and one control sample or vice versa.

Curing. Inoculated and control curing jars were incubated at room temperature for 3 days. During this time, the bacterial count increased about 7-fold in jars in which inoculum had been added. In one exceptional experiment, the bacterial count of the inoculated brine increased from 200,000,000 to 2,600,000,000 per ml, the peak count during the 3-day curing period. The peak was reached after 48 hr. This amount of increase was determined by plate counts of brine made immediately after addition of the inoculum and at 24-hr intervals during the curing period. Control curing brines were tested for sterility and treated brines for purity of culture by culturing at the end of the curing period. From each well mixed brine, transfers of 1 ml were made to veal infusion agar plates and to fluid thioglycolate medium. A direct smear from each brine stained by Gram's method was examined. Specimens which failed to meet sterility specifications by these criteria were discarded.

Preparation of materials for organoleptic testing. Following the curing period, meat for taste testing was removed from the brines, rinsed quickly in tap water and ground twice in a sausage grinder. Half of the treated meat and half of the control meat were extracted in distilled water (640 g per L) at approximately 5 C for 48 hr and half of each lot cooked in cakes by steaming for 15 min in sufficient distilled water to cover and evaporating to palatable moisture content. The extracted meat was removed from the broth by filtering through gauze, the extract diluted with an equal volume of distilled water, boiled for 5 min, decanted from the coagulated material, and distributed in serological test tubes for tasting. Brine, both control and inoculated, was diluted to approximately the same degree of saltiness as the diluted meat extract, boiled for 5 min, decanted from coagulated material, and tubed for testing.

Organoleptic testing. Organoleptic testing of brine, meat, and meat extract was done by a carefully selected panel (McLean *et al*, 1959) with demonstrated ability to repeat their judgments (Bradley, 1953). Triangular testing was used and data were analyzed for significance according to tables by Roessler *et al.* (1948).

Histological preparations. In one experiment, samples of cured meat 2 by 8 mm were cut from the center of chunks of ham, control and inoculated, and fixed in 10 per cent formalin. Paraffin sections 5 and 10 μ in

thickness were prepared for microscopic examination with Giemsa staining (Strumia, 1936).

Results and Discussion

Results are recorded in table 1 and show that, for the three materials tested, the bacterium added to curing brine produced a distinctive flavor. In the three experiments reported, the number of tasters correctly matching unknowns was sufficient, except in one case, to make the results fall within the highly significant range, that is, within the 0.001 level (1 in 1000 chances of error) indicating that a difference had been produced (Roessler *et al.*, 1948). In one experiment, a majority of the members of the taste panel preferred meat cured in sterile brine to that cured in brine supporting active growth of the bacterium. No bacteria were found within the tissues in the inoculated meat when paraffin sections with Giemsa staining were examined. Material from one out of three identical experiments (15 sections from control block and 15 from treated block) was considered adequate for this conclusion. Therefore, the changes produced by bacteria resulted from the passage of bacterial metabolites from the brine into the meat.

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Summary

The data presented establish that, under the experimental conditions employed, a species of *Pseudomonas*, strain HB28, growing in a meat curing brine affected flavor. In addition, methods have been evolved for evaluating microorganisms for flavor production in meat curing brines in a completely controlled system.

REFERENCES

Bradley, R. A. 1953 Some statistical methods in taste testing and quality control. Biometrics, **9**, 23.

Brady, D. E., Peterson, W. J., and Shaw, A. O. 1944 Riboflavin and thiamine contents of pork loin muscles and their retention during cooking. Food Research, **9**, 400–405.

Brooks, J., Haines, R. B., Moran, T., and Pace, J. 1940 The function of nitrate, nitrite and bacteria in the curing of bacon and hams. Great Britain Food Investigation Board, Special Report, 47–59.

Deibel, R. H. and Niven, C. F., Jr. 1957 Studies on the microflora of commercial ham curing brines and their significance in curing. Proc. 2nd Intern. Symposium on Food Microbiology, Cambridge, England.

Gibbons, N. E. 1939 Microbiology of meats. Rept. Proc. 3rd Intern. Congr. Microbiol., 702–703.

Jacobson, Marion and Fenton, Faith 1956 Effects of three levels of nutrition and age of animal on the quality of beef. I. Palatability, cooking data, moisture, fat and nitrogen. Food Research, **21**, 415–440.

Jensen, L. B. 1954 *Microbiology of meats*, 3rd ed. Garrard Press, Champaign, Illinois.

McLean, R. A., Gaddis, A. M., Suggs, F. G., and Crowe, P. F. 1959 Evaluation of flavors in meat by the use of aqueous extracts. Food Technol. In press.

Roessler, E. B., Warren, J., and Guymon, J. F. 1948 Significance in triangular taste tests. Food Research, **13**, 503–505.

Stanier, R. Y. 1947 Acetic acid production from ethanol by fluorescent *Pseudomonads*. J. Bacteriol., **54**, 191–194.

Strumia, M. M. 1936 Rapid universal blood stain; May-Gruenwald-Giemsa in one solution. J. Lab. Clin. Med., **21**, 930–934.

Tanner, F. W. 1944 *The microbiology of foods*, 2nd ed. Garrard Press, Champaign, Illinois.